NUCLEIC ACID PURIFICATION

CROSS-REFERENCE TO RELATED APPLICATIONS

This application is a continuation of U.S. patent application Ser. No. 14/728,862 filed Jun. 2, 2015 which is a divisional of U.S. patent application Ser. No. 13/025,923, filed Feb. 11, 2011 now issued as U.S. Pat. No. 9,174,210, which is a continuation of U.S. patent application Ser. No. 12/699,564, filed Feb. 3, 2010, now issued as U.S. Pat. No. 9,012,208 and claims the benefit of the filing dates of U.S. Provisional Application Ser. No. 61/206,690, filed Feb. 3, 2009; and No. 61/207,017, filed Feb. 6, 2009. Each of the preceding are hereby incorporated by reference in their entireties.

STATEMENT OF GOVERNMENT INTEREST

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BACKGROUND OF THE INVENTION

A. The Unmet Need—Unprocessed Clinical and Forensic Samples

From the first isolation of nucleic acids by Miescher and Altmann in the second half of the nineteenth century (Miescher, Friedrich (1871) "Ueber die chemische Zusammensetzung der Eiterzellen," in F. Miescher. Die Histochemischen and physiologischen Arbeiten Vol. 2:3-23) to the most sophisticated molecular biological techniques available today, the process of DNA extraction has been streamlined substantially. Nevertheless, there is a pressing need in the clinical, biothreat detection, and forensics communities for sensitive, robust, and reliable integrated methods of DNA purification that are rapid, cost-effective, and neither labor- nor space-intensive. In particular, there is an unmet need for methods and devices that can rapidly purify nucleic acids from unprocessed clinical or forensic field samples without any manual handling or processing.

Ideally, novel methods for nucleic acid purification are needed to address the numerous and varied existing and emerging markets for delivering genomic information, particularly the delivery of genomic information in the field, and for point-of-care and near point-of-care applications. For example, in the field of human identification, there is an unmet need in the forensic community to be able to generate a DNA fingerprint rapidly, whether in the laboratory or in the field (e.g. at borders, ports of entry, the battlefield, and military checkpoints).

Similarly, in order to protect civilian and military populations, it is critical to improve the identification of environmental biothreats. More rapid, more sensitive, more specific, and more detailed identification will allow improved strategic and tactical responses by civilian and military authorities, and more effective remediation activities. The rapid application of nucleic acid analysis technologies including nucleic acid amplification, hybridization, and sequencing can provide critical information in this regard.

Furthermore, the ability to rapidly diagnose clinical infections (whether caused by biothreats or conventional pathogens) would have a profound impact on society. For example, drawing a blood sample from a septic patient and determining both the identity of the pathogen or pathogens as well as their antibiotic resistance profiles based on nucleic acid analyses within an hour or less would allow specific antimicrobial therapy to begin immediately (the analogous situation for viral diagnostics and drug resistance profiles is also critically important). The ability to rapidly generate nucleic acid analytic information from clinical samples would also have substantial impact on the diagnosis and treatment of a wide range of diseases ranging from cancers to immune system disorders; essentially every category of diseases would be impacted. The same approach could also be applied to pharmacogenomics, the use of genetic information to predict the suitability of a given pharmacologic intervention.

B. Prior Art Approaches to DNA Purification

The basic approach to extraction and purification of nuclear DNA from mammalian cells was developed over three decades ago (N. Blin, D. W. Stafford (1976). A general method for isolation of high molecular weight DNA from eukaryotes. *Nucleic Acids Res.* 3(9): 2303-8) and has two major steps: the lysis of the cell types of interest and the purification of DNA from other cellular components in solution (particularly proteins) and cellular and tissue debris. Cell lysis and (when appropriate) DNA solubilization can be accomplished by mechanical (reviewed in J. Brent (1998). Breaking Up Isn't Hard To Do: A cacophony of sonicators, cell bombs and grinders" *The Scientist* 12(22):23) and non-mechanical techniques. Simple mechanical approaches include the use of a blenders and homogenization by forcing cells through restrictive openings. Sonication is based on the exposure of cells to high-frequency sound waves, and bead approaches are based on exposing cells to violent mixing in the presence of various beads.

Chemical disruption of cells is an alternative to mechanical disruption. Detergents are important chemical lytic agents that act by disrupting lipid bilayers. Additional properties of detergents may allow protein structure to be maintained (e.g. zwitterionic and nonionic detergents) or disrupted (ionic detergents). Sodium dodecyl sulfate (SDS), an ionic detergent, is commonly used in forensic DNA extraction protocols due in part to its ability to solubilize macromolecules and denature proteins within the cell (J. L. Haines et al (2005) Current Protocols in Human Genetics Vol. 2, (2005 John Wiley and Sons, Inc. Pub.). Proteinase K is often used in tandem with detergent-based (e.g. SDS, Tween-20, Triton X-100) lysis protocols. Another form of detergent lysis is based on FTA paper (L. A. Burgoyne (1997) Convenient DNA Collection and Processing: Disposable Toothbrushes and FTA Paper as a Non-threatening Buccal-Cell Collection Kit Compatible with Automatable DNA Processing, 8th International Symposium on Human Identification, Sep. 17-20, 1997 Orlando, Fla.; G. M. Fomovskaia et al., U.S. Pat. No. 6,958,392). This is a cellulose filter impregnated with a weak base, an anionic detergent, a chelating agent, and preservatives.

In the case of a clinical or environmental sample, a critical first step towards nucleic acid analysis is the isolation or purification of some or all of the nucleic acid present in the sample. The biological material in the sample may be lysed and nucleic acids within the lysate may be purified prior to further analysis. Alternatively, nucleic acids contained within the lysate may be analyzed directly (e.g. Phusion Blood Direct PCR kit (Finnzymes, Espoo, FN) and Daniel et al., U.S. Pat. No. 7,547,510).

As those skilled in the art will recognize, purifying nucleic acids from unprocessed clinical, environmental, or